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Distribution and Importance of Citrus Leaf and Fruit Spot Disease (Pseudocercospora Angolnesis) in North-Western Ethiopia

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Abstract

This study was proposed to assess the distribution and importance of citrus leaf and fruit spot disease in North Western Ethiopia. The result showed that on sweet orange the prevalence of the disease was ranged from (0 to 100 %). Disease incidence was ranged from (0 to 86.48 %), and from (0 to100 %) on leaves and fruits, respectively. Disease severity was ranged from (0 to 24.18%) on leaves, and from (0 to 73.0 %) on fruits.

Correlation analysis was carried out for both disease incidence and severity as response variables and altitude, average daily temperature, mean annual rainfall and tree age as predictor variables. Linear correlation was detected only between mean annual rainfall and disease severity and incidence of leaves and fruits. The regression analysis result indicated that mean annual rain fall has a significant effect over disease severity and incidence of leaves and fruits at ($P \leq 0.05$).

Keywords: Pseudocercospora Angolensis, Prevalence, Leaf and Fruit Spot, Incidence

Introduction

Citrus currently is cultivated in more than 140 countries, mainly located between 35° south and northern latitudes. However, the major commercial production areas are in the Sub-Tropics where the finest quality citrus fruits are produced [13]. Citrus is a major source of cash and nutrition in many developing countries [25]. In Africa, the citrus industry is relatively a recent development [14]. Where, the lead-producing countries at a commercial scale are Egypt, South Africa, Morocco, Algeria, and Tunisia [10]. However, in Tropical Africa, citrus has been grown largely by smallholder farmers for domestic consumption and export [14].

Citrus is among the most important fruit crops in Ethiopia. And its cultivation was started in upper Awash Valley and Melkassa areas that are located in the central parts of the country [11]. However, its production and productivity are seriously threatened by various diseases including leaf and fruit spot disease [22, 15, 23].

Leaf and fruit spot disease of citrus is a critical threat to citrus production in Ethiopia due to its impact on yield, exchange of materials, and citrus trade [16]. In some of the affected areas, farmers reported entire fruit loss and were compelled to abandon their citrus trees [8, 15].

In Ethiopia because of the lack of appropriate option that can be used to manage the disease, its spread to new areas where the disease was not detected before and the extent of damage caused by the disease have been increasing in an alarming situation. As a result, diagnosis and regular monitoring of the disease is required for devising sustainable disease management practices thereby reducing the yield loss and improving the quality of citrus fruits [16]. Therefore, this study was carried out to assess the distribution and status of citrus leaf and fruit spot disease in North-Western Ethiopia.

Materials and Methods

Survey Area

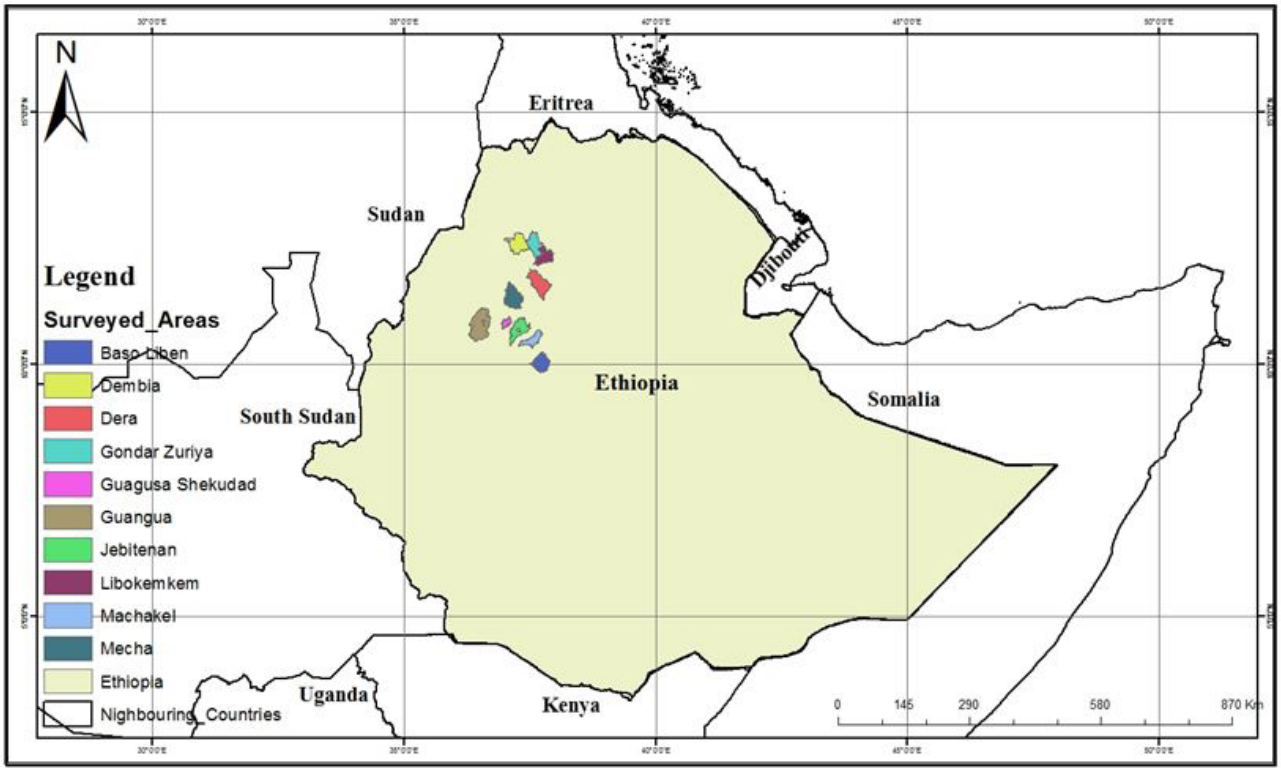

Figure 1: Map of the surveyed areas

The field survey was carried out in the major citrus growing areas of North Western Ethiopia to evaluate and quantify the prevalence, incidence and severity of citrus leaf and fruit spot disease, during 2020/21. And the locations of the surveyed areas fall between longitudes of 37°35'37" E and latitudes of 11° 27'21" N (Figure 1).

Sampling and Sampling Units

A purposive sampling method was implemented to select the assessed orchards. Based on availability, a total of 20 orchards were selected. Sweet orange, Mandarin and Lemon were the evaluated citrus species. In each selected orchard based on availability, five to ten trees of sweet Orange, Mandarin, and Lemon were chosen randomly diagonally across the field to determine disease incidence and severity of leaves and fruits [19, 15]. And each sampled tree was considered as a sample unit.

Disease Assessment

Disease prevalence was determined by the number of orchards surveyed showing the disease symptom, expressed as the percentage proportion of the total number of orchards surveyed [2].

And disease incidence was estimated both on leaves and fruits of the plant. On leaves, it was estimated by counting the visibly infected and total number of leaves on eight randomly selected terminal shoots from the upper and lower halves of the canopy in four directions (North, South, East, and West) of each selected tree, and expressed as a percentage [3]. It was computed by [18] formula as:

$$\text{Disease incidence on leaves } (\%) = \frac{\text{no of leaves infected per tree}}{\text{Total no of assessed leaves per tree}} \times 100$$

On fruits, it was assessed on 5 to 40 randomly selected intact fruits in four directions of each tree based on the presence or absence of visible disease symptoms on each fruit, depending on availability [3]. And it was calculated by using the [16] formula:

$$\text{Disease incidence on fruits } (\%) = \frac{\text{noof infected fruits per tree}}{\text{Total no of assessed fruits per tree}} \times 100$$

Disease severity was assessed on the same leaf and fruit samples that were chosen for disease incidence scoring. On leaves, it was estimated based on a zero-to-four scoring scale, where 0 = no symptoms, 1 = 1 to 25%, 2 = 26 to 50%, 3 = 51 to 75% and 4 = above 75% of leaf area infected [1, 9].

And on fruits, it was recorded using the following zero to four scoring scale, where 0 = healthy, 1 = less than 5%, 2 = 5 to 20%, 3 = 21-50% and 4 = above 50% of fruit surface affected [20].

For analysis purposes, severity grades were converted into percentage severity index (PSI) and it was calculated using the following formula suggested by [6]:

$$\text{PSI} = \frac{\text{Sum of all numerical ratings}}{\text{Total no. of observations} \times \text{Maximum disease score}} \times 100$$

During the survey, to obtain further information about the status of citrus production and management practices of the sampled areas, a questionnaire was used. The questionnaire mainly compiled: the farm size, the number of citrus trees, type of citrus species and cultivars, orchard and/or tree age, soil type, input application, pest infestation history and management practices implemented and ownership. All information collected were summarized and described to give an overview of citrus production in North Western Ethiopia. A face-to-face (Personal) interview method was implemented. And depending on the type of ownership the interviewee were Farmers, Investors, and Agronomy experts.

Sample Collection

In each orchard, infected leaf and fruit samples were taken from selected sweet orange, mandarin, and lemon trees showing the typical symptom of the disease. And samples were placed in transparent plastic bags, covered with brown paper bags, labeled properly, kept in cool icebox containers, and then transported to the laboratory for isolation, purification, and identification of the causative pathogen.

Isolation and identification of the Pathogen

In the laboratory to disinfect the working environment and essential tools for the work, initially wiping of the work area with 70% ethyl alcohol and dipping of the laboratory instruments such as; Forceps, Scalpels, Needles, and Knives in 70% ethyl alcohol and flame drying of them were done frequently. The same procedure was also repetitively applied at the end of every laboratory session.

Then leaf and fruit samples were washed in tap water and surface sterilized in 70% ethanol each for one minute, and then rinsed three times with distilled water was carried out. Sterilized leaves and fruit peels were cut, and four to six-leaf discs and peel pieces of fruits were placed on each Petri dish containing potato dextrose agar (PDA) in three replicates and incubated at 25±1°C, under the light. After five days of incubation suspected fungal colonies were developed. Then with these colonies, purification was done using hyphal tipping onto fresh PDA medium and incubated for four to seven days at 25±1°C and was allowed to sporulate. And the sporulated cultures were examined under binocular microscope supported with cell sense entry soft were for identification, based on morphological characters of somatic and reproductive structures including spores/conidia and spore-forming structures. On the observed characteristics, confirmation was made with those available in the manuals of Barnett and Hunters [4]. Then to preserve the pathogen for future use, the agar deep culture tube inoculation method was implemented. That is, the pathogen was delivered using a needle and stabbed deep down the center of 1% water agar. The inoculated culture tubes were kept in a refrigerator at 4 °c.

Pathogenicity Test

A pathogenicity test was carried out to confirm the isolated organism as a pathogen using Koch's postulates on commonly grown sweet orange, mandarin and lemon citrus species. The test was implemented on young and apparently healthy detached leaves of the same species from which the pathogen was isolated.

The detached leaves were washed in distilled water; surface sterilized with 70% ethanol and rinsed repeatedly with sterile distilled water.

In each Petri dish containing water agar (1%), two sterilized leaves of each species were placed, by keeping the lower side up. Then inoculation of leaves was carried out by placing drops of conidial suspension, with a concentration of 10^5 to 10^6 conidia mL-1[8, 20] by placing a drop of spore suspension on leaves. The Petri dishes were enclosed with Para film to maintain high relative humidity that can facilitate the infection and significantly increase the success rate of the test. Whereas, controls were maintained using only distilled water in place of inoculum suspension. Cultures were incubated at 26°C for two weeks, and inoculated leaf samples were regularly observed for the appearance of disease symptoms.

Then the re-isolation procedure was carried out from newly inoculated leaves to demonstrate Koch's postulate. And the re-isolated cultures were examined for cultural and morphological comparisons with the original cultures to confirm that it is the same pathogen we have.

Statistical Analysis

SPSS (Statistical Product and Service Solution), version 20.0 [24] was the statistical package used for analysis.

Results

Distribution and Status of Citrus Leaf and Fruit Spot Disease During 2020/21

Based on availability, 280 citrus trees (200 sweet oranges, 40 mandarins, and 40 lemons) that were grown in 10 major citrus growing districts were examined. And the result showed that in the studied areas leaf and fruit spot was the most prevalent disease of citrus. The mean prevalence rate of the disease was (80%). The highest disease prevalence (100%) was recorded in eight districts (Gondar Zuria, Derra, Mecha, Jabitehinan, Basoliben, Machakel, Ayehugugusa and Guangua) Whereas, it was null in two districts (West Dembia and Libokemkem). The reason for high prevalence of the disease could be attributed due to the presence of conducive environmental conditions for the pathogen particularly high rain fall and relative humidity. This result was consistent with the former report [21]. According to the report, the development and spread of Pseudocercospora angolensis is favored in areas with high rain fall and humidity. Besides, the most possible reason could be shortage of disease resistant varieties, lack of fertilization, moisture stress and poor agronomic practices. Whereas, the reason for the absence of the disease in two districts could be attributed to the absence of inoculum source that could be brought by wind, infected fruits and propagating materials. Similarly, [19, 12, 17] also reported that inadvertent transport of infected fruits and propagating materials from infected areas can play some role in the spread of the fungus.

Severity and Incidence of Citrus Leaf and Fruit Spot Disease in 2020/21

Citrus leaf and fruit spot disease incidence was ranged from (0 to 86.48 %), and from (0 to100 %) on leaves and fruits, respectively. And disease severity was ranged from (0 to 24.18%) on leaves, and from (0 to 73.0 %) on fruits. Variations among districts were observed in their recorded disease incidence and severity rate. This variation could be attributed to differences among districts in their received amount of mean annual rain fall. This result was consistent with the former reports [12, 15, 5]. According to the reports, disease incidence varies with the amount of rainfall.

On leaves, the highest disease incidence (86.48%) and severity (24.18%) were recorded in Mecha district. And on fruits, the highest disease incidence (100%) was observed in Ayehuguagusa and Guangua. And it was in Jabitehinan where the highest disease severity (73%) was discerned (Figure 2). This variation could also be attributed to differences among districts in their received amount of mean annual rain fall. [15] Also reported that citrus that are grown in areas with high moisture and humidity were severely affected.

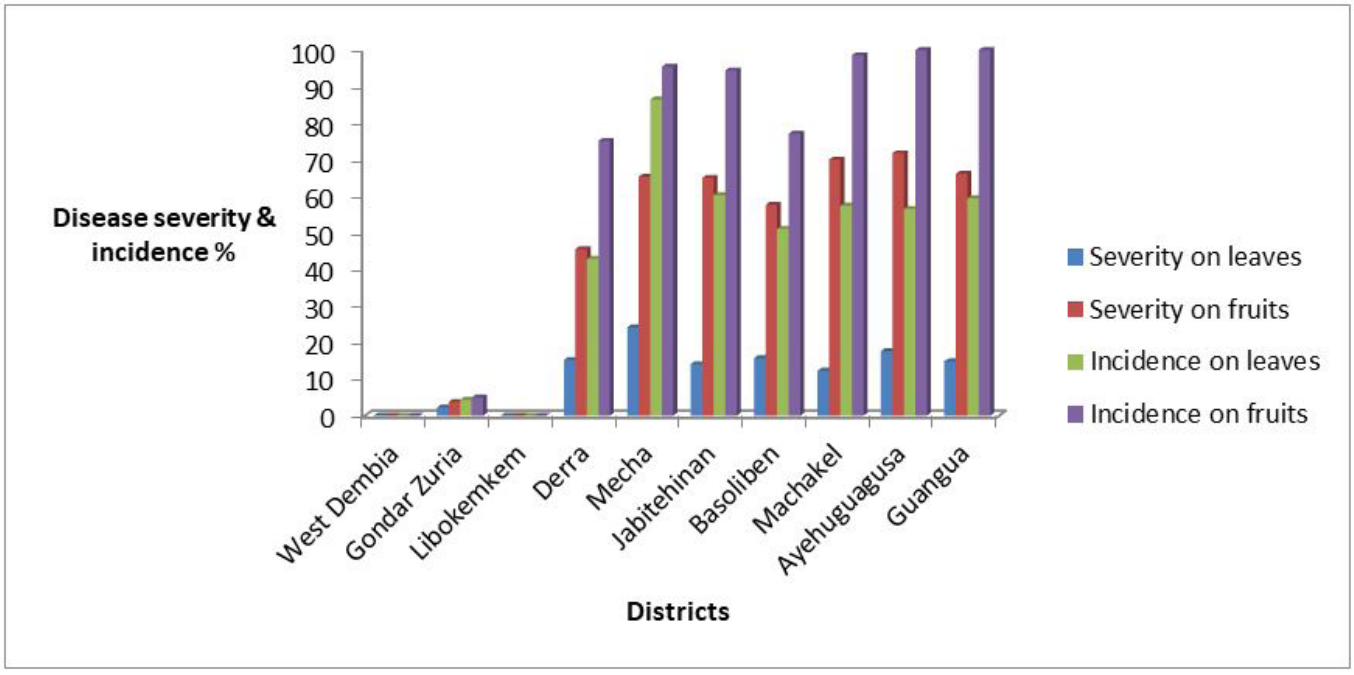

Figure 2: Severity and incidence of citrus leaf and fruit spot disease across districts in 2020/21

Several small-scale citrus growers have abandoned their trees due to severe infection of citrus leaf and fruit spot disease [25]. Such trend was detected in this study as well i.e., in areas where the highest citrus leaf and fruit spot disease infection were recorded, there was no citrus fruit harvest at all. As a result of this, citrus growers were exercising uprooting of their citrus trees and replacing them by other fruit crops (Mango and Avocado).

Correlation and regression analyses were carried out for both disease incidence and severity as response variables and altitude, average daily temperature, mean annual rainfall and tree age as predictor variables. The result revealed that linear correlation was detected only between mean annual rainfall and disease severity and incidence of leaves and fruits (Table 1). As a result, regression analysis was conducted on those explained and predictor variables that showed linear relationship. And the result indicated that mean annual rain fall has a significant effect over disease severity and incidence of leaves and fruits at ($P \leq 0.05$) level.

Explained variables	Predictor variables			
	Altitude	Mean annual rainfall	Average daily temperature	Tree age
Severity on leaves	- 0.08	0.71*	- 0.17	0.04
Severity on fruits	0.01	0.61*	- 0.22	0.06
Incidence on leaves	- 0.04	0.68*	- 0.09	0.02
Incidence on fruits	0.0	0.62*	- 0.19	0.04

*Correlation is significant at $P \leq 0.05$ level

Table 1: Pearson's linear correlation analysis output between explained and predictor variables in 2020/21

Identified Agronomic and Pest Management Constraints of the Studied Areas in 2020/21

In the surveyed areas beside citrus leaf and fruit spot disease high insect pest predominantly scales and leaf miners (100%) infestation were observed and caused a devastating effect both in the productivity and production of citrus fruit. However, to resolve the problem there was no insect pest management effort made in all assessed orchards (100%, or 20 out of 20). Besides, moisture stress as a result of absence or low irrigation schemes, lack of improved agronomic practices (pruning, weeding and clearing) and shortage of better-quality citrus cultivars were contributed a lot to the existence of low citrus fruit productivity and production as well.

According to the information obtained from the interviewee, chemical fertilizer application was practiced only by the government-owned orchards (20%, or 4 out of 20). And application of animal manure was practiced only by small-scale farmers-owned orchards (80%, or 16 out of 20).

Pathogenicity Test

Chung and Timmer (2009) found that Pseudocercospora angolensis can be isolated and cultured from lesions of infected leaves or fruits and can grow well and sporulate properly on various artificial culture media. Such trend was detected in this study as well i.e., to confirm the pathogenicity of the identified isolates of Pseudocercospora angolensis on its host, Koch's postulates were implemented. And among the tested pure isolates of the pathogen (81.25%), (66.66%) and (41.66%) of inoculated sweet orange, mandarin and lemon leaves, respectively were caused typical symptom of the disease (Table 2). However, there was no symptom observed on water inoculated controls.

Species	No of inoculated leaves	No of infected leaves	Infection percentage
Sweet orange	48	39	81.25
Mandarin	12	8	66.66
Lemon	12	5	41.66

Table 2: Inoculation success of conidial suspension of Pseudocercospora angolensis

Discussions

The present survey result showed that leaf and fruit spot was the most predominant disease of citrus in North Western Ethiopia. Visible variations among surveyed districts were observed in their recorded disease incidence and severity rate. And this variation could be attributed to differences among districts in their received amount of mean annual rain fall. Among predictor and explained variables that undergone correlation analysis, it was only the amount of mean annual rain fall that showed linear correlation. Consequently, regression analysis was conducted on those explained and predictor variables that showed linear relationship. And the result indicated that mean annual rain fall has a significant effect over disease severity and incidence of leaves and fruits at ($P \leq 0.05$) level.

This study also confirmed that there was no citrus fruit harvest carried out in areas where the highest citrus leaf and fruit spot disease infection were recorded. As a result of this, citrus growers were exercising uprooting of their citrus trees and replacing them by other fruit crops (Mango and Avocado).

Conclusions

This study result revealed that citrus fruit production is seriously threatened by citrus leaf and fruit spot disease in most of the areas addressed by this study. Besides citrus leaf and fruit spot disease, insect pest infestation, moisture stress, lack of improved agronomic practices, shortage of better-quality citrus cultivars, lack of fertilization and growing of long- standing citrus trees were some of the identified constraints that contributed a lot for the existence of no/ low citrus fruit production as well.

Therefore, from the results of the present study to provide sustainable citrus fruit productivity and production in the study areas; effective and efficient citrus leaf and fruit spot management strategies should be implemented. Furthermore, in order to get full illustrates of the distribution and importance of citrus leaf and fruit spot disease, it is useful, to conduct related disease surveys in major citrus growing areas of the Region.

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Supplementary Information

Description of the Geographic Locations, Climatic Conditions and Tree Ages, During 2020/21

No	Districts	Altitude (m.a.s.l)	Daily average temperature (^{0}c)	Mean annual rainfall (mm)	Soil type	Tree age (Years)
1	West Dembia	2050	30.0	1100	Sandy	23
2	Gondar Zuria	2065	22.5	1150	Brown	30
3	Libokemkem	2325	19.5	1100	Brown	21
4	Derra	2080	21.5	1250	Sandy loam	21
5	Mecha	2150	25.5	2000	Brown	25
6	Jabitehinan	1900	23.0	1250	Red	21
7	Basoliben	1650	19.0	1050	Sandy loam	21
8	Machakel	2650	22.5	1350	Sandy loam	25
9	Ayehuguagusa	2470	19.0	2000	Sandy loam	30
10	Guangua	1750	25.0	1650	Sandy	25